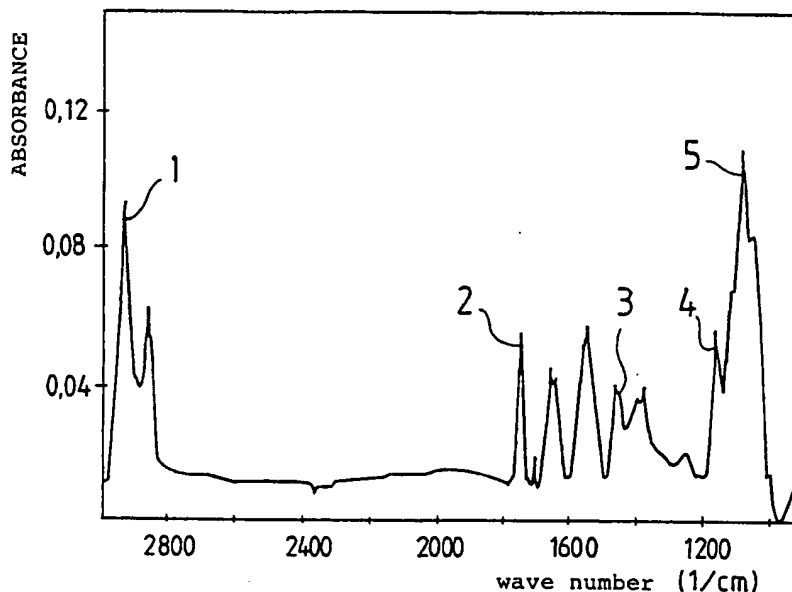




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(54) Title: METHOD FOR QUANTITATIVE DETERMINATION OF FAT IN AN EMULSION WHICH CONTAINS FAT PARTICLES



(57) Abstract

The invention relates to a method for quantitative determination of fat from an emulsion, such as milk or a milk product, containing fat particles. The method uses an IR absorption technique in which the determination is based on a specific absorption peak in the absorption spectrum of the emulsion sample, and the essential idea of the invention is that the absorption peak, at a wave number of approx. 1160-1190 (1/cm), of the single carbon-oxygen bonds of the ester groups of the fat molecules is used in the determination. When the determinations are carried out on milk or on a milk product, the effect of lactose can, when so desired, be taken into account by measuring the characteristic absorption peak of lactose and by carrying out, on the basis of it, a correction in the fat concentration value yielded by the C-O absorption peak. Since scatter does not affect the absorption peak of the C-O bond, the determinations according to the invention can be carried out directly from full milk, without a preceding homogenization treatment.

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Method for quantitative determination of fat in an emulsion which contains fat particles

The present invention relates to a method for quantitative determination of fat in an emulsion which contains fat particles, in which method the infrared absorption of the emulsion sample is measured and the fat is determined on the basis of a specific absorption peak in the absorption spectrum by eliminating from it any error effect possibly caused by the other constituents of the emulsion.

The principal targets of quantitative fat determinations are milk and products made from milk. The natural fat concentration in milk is usually within the range 3.0-5.5 %, but the fat concentrations in different milk products may vary from close to 0 to more than 40 %. Measurement of the fat concentration is required as a basis for the milk price to be paid to the producer and additionally in the quality control of products prepared from milk.

The determination of the fat concentration in milk is possible by traditional chemical means, of which so-called Gerber and Röse-Gottlieb methods can be mentioned as examples. However, infrared absorption is currently used in routine determinations, although chemical methods continue to be necessary for the calibration of the IR analyzers. Most commonly the absorption peak, at a wave number of approx. 1750 (1/cm) (at a wavelength of approx. 5.7 μm), of the carbon-oxygen double bonds of the triglyceride part contained in the fat molecule is used in IR determinations. In addition to this there is used a method described in EP Patent publication 0012492, which is based on the absorption peak, at a wave number of approx. 2870 (1/cm) (at a wavelength of approx. 3.5 μm), of the carbon-hydrogen bonds in the fat molecule.

The last-mentioned method, based on the absorption of the C-H bond, has been justified by the fact that the method, contrary to the method based on the C=O double bond, is not dependent on variations in the fat composition of milk, which is due to the fact that the amount of the absorbing C-H bonds in the fat molecule is directly proportional to the size of the molecule. On the other hand, when the C-H bond is used, it is necessary to take into account the contribution to the absorption peak by other substances present in milk, mainly proteins and lactose, which contain C-H bonds. This can be done by measuring, in addition to the absorption peak of the C-H bond also the IR absorption peaks characteristic of the said other substances and by calculating on the basis of them a correction of the C-H bond absorption peak, thereby arriving at the fat concentration aimed at in the determination.

The determination of the fat concentration in milk on the basis of the absorption of the C-H bond does, however, involve problems which are more difficult than the above-mentioned computational correction of the error caused by proteins and lactoses. The first problem to be mentioned is that the error-causing milk constituents which contain C-H bonds are by no means limited to proteins and lactose; dozens of them are present in milk, at concentrations which vary with great random. Some of such constituents are vitamins, nucleic acids, esters of organic acids, lactones, amino acids, amines, and cholesterol. The concentrations of these substances are dependent, among other things, on the breed and state of health of the cow, on its feed, which varies from one season to another, and on the condition of the sample at the time of the analysis, which is dependent on the storage of the sample. The concentration of each individual substance in milk remains usually so low that taking it into account in the determination of fat in the same manner as proteins and lactose is not possible in practice, but the combined total concentration of these substances in milk may rise up to 0.1 %, and in poorly stored samples even higher.

than this, in which case they will cause an error of a corresponding order of magnitude in the fat concentration calculated from the C-H absorption peak.

Another problem associated with the use of the C-H absorption peak is that the peak is located in the infrared spectrum inconveniently on the slope of a strong absorption peak of water, from which it is difficult to distinguish. Furthermore, the proportion of water in the sample on which the determination is being performed is not always known precisely, which causes in the results an uncontrolled error which is further multiplied in the corrections calculated for proteins and lactose.

A further error source which hampers the use of the C-H bond is the scatter occurring in the sample alongside IR absorption. The scatter caused by fat particles is inversely proportional to the fourth power of the wavelength used, and is additionally dependent on the ratio of the wavelength to the diameter of the fat particles in such a manner that, when the wavelength increases to a value higher than the particle diameter, the scatter rapidly decreases to an insignificant level. Scatter thus constitutes a problem in milk fat determination when the wavelength of the absorption peak used corresponds to or is smaller than the diameter of the particles. When the diameter of the fat particles in raw milk is in the order of 0.5-5 μm , it follows from this that scatter strongly disturbs C-H bond absorption occurring at a wavelength of 3.5 μm , unless the fat particles can be reduced to a diameter clearly less than 3.5 μm , preferably to a diameter less than 2 μm , in a homogenization before the fat determination. Therefore it is stated in the above-mentioned EP patent publication 0012492 that the mean diameter of the fat particles may be at maximum 2 μm , and usually the IR analyzers used for milk fat determinations are indeed equipped with homogenizers by means of which the fat particles can be chopped to a size range of 0.5-1.5 μm . However, in continuous use the homogenizer is subject to gradual

wear, whereby the dimensions of its mechanical parts are changed and its operating capacity deteriorated to the point that, with time, more and more poorly homogenized milk samples, containing also fat particles larger than $2\text{ }\mu\text{m}$ in diameter and giving erroneous results, arrive in the measuring cuvette. The problem could be in part diminished by, for example, homogenizing the sample several times, but this would lower the analyzer capacity to such an extent that the system is not practicable.

When milk fat determination is based on the absorption peak of the C=O double bond, the problem of scatter is clearly smaller. This is due to the absorption peak being located at a higher wavelength, $5.7\text{ }\mu\text{m}$, which exceeds the maximum size of fat particles, which is approx. $5\text{ }\mu\text{m}$. The absorbance curve depicting the concentration of fat as a function of absorption is clearly more gently sloping with the C=O absorption peak than with the C-H absorption peak, and respectively the precision of the determination is higher, especially at higher concentrations of fat. However, also when the C=O double bond is used, obtaining precise results presupposes the homogenization of the milk before the measuring.

The object of the present invention is to provide a new method for quantitative determination of fat by an infrared absorption technique in which the above-mentioned problem of scatter and also the disadvantages associated with the use of the C-H bond are avoided. The method according to the invention is characterized in that the determination is made from an absorption peak, at a wave number of approx. $1160\text{--}1190\text{ (1/cm)}$, of the single carbon-oxygen bonds (C-O) of the ester groups of the fat molecules.

The wave number, approx. $1160\text{--}1190$, of the IR absorption peak used in accordance with the invention corresponds to a wavelength of approx. $8.4\text{--}8.6\text{ }\mu\text{m}$, which is so much above the maximum size, approx. $5\text{ }\mu\text{m}$, of fat particles that scatter will no

longer affect the result of the determination. A comparison of the previously used C-H and C=O absorption peaks with the C-O absorption peak used according to the present invention shows that the effect of scatter on the C-H peak is 34-fold and on the C=O peak five-fold as compared with the C-O peak. This means that, in the method according to the invention, the homogenization of milk before the absorption measurement is unnecessary. The measurement can be performed from raw milk, in which mere careful mixing suffices for providing a representative sample.

From the elimination of the homogenization step there follows the advantage that the IR analyzer may be simpler in structure than previously. A homogenizer typically made up of a hole and a ball spring-actuated against it, and the high-pressure pump required for it, can be omitted, and a conventional pump operated at low pressures suffices for the feeding in of the sample. As the apparatus is simplified, also its maintenance requirement is reduced, and as the problems due to the wear of the homogenizer have been eliminated, the results obtained are more reliable than previously.

The absorption peak, at a wavelength of approx. 1160-1190, of the C-O bond, used according to the invention, is located in the IR absorption spectrum at a place considerably more advantageous with respect to the absorption of water than is the absorption peak of the C-H bond. When milk or some other water-based emulsion is measured, the reading of the peak is therefore easier and the determination result obtained is not dependent on the amount of water present in the sample.

It is also a significant advantage of the invention that the C-O absorption peak used is highly specific with respect to fat. With the exception of certain esters of amino acids or other organic acids, present incidentally in small amounts, milk or milk products do not contain any constituents having a

single C-O bond other than fat. The single C-O bond is superior in specificity even over the C=O bond, which is present in free amino acids and other organic acids which are present in milk in larger amounts than are the above-mentioned esters. This means that, in the method according to the invention it is not necessary to use the computational compensations which are necessary in the determination based on the C-H bond and in which the contribution of proteins and lactose to the C-H absorption peak must be taken into account. The only slight correction requirement in the determination of the fat concentration in milk by the method according to the invention is due to a lactose peak which is located close to the C-O absorption peak and may slightly overlap with the C-O peak, primarily owing to deficiencies in the resolution of the analyzers. However, the error in the result will not be great even if the lactose peak is not taken into account.

In addition to measuring the absorption peak of the C-O bond it is necessary, regardless of the type of the emulsion, to measure the absorption at a reference wave number, which may be, for example, a wave number of approx. 1200 or approx. 1300-1350. For this reason it may be preferable to measure a continuous infrared absorption spectrum within a somewhat wider wave number range, such as a range of approx. 1160-1350.

When determination is performed on milk or on a milk product, for example, unhomogenized milk which has a fat concentration of approx. 3.0-5.5 % and in which the fat particle size varies mainly within the range 0.5-5 μm , it is possible according to the invention to measure, besides the C-O absorption peak, also the characteristic absorption peak of lactose at a wave number of approx. 1050, in which case it is possible, on the basis of the latter, to eliminate the effect of lactose on the C-O absorption peak. In this case it is preferable to measure a continuous IR absorption spectrum within a wave number range of approx. 1050-1350.

In the accompanying drawing,

Figure 1 depicts an infrared absorption spectrum of full milk (fat concentration 3.4 %) measured by means of a Mattsson FTIR spectrometer within a wave number range of approx. 1000-3000, and

Figure 2 depicts an infrared absorption spectrum of the triglyceride ester of oleic acid, measured by means of the same spectrometer within a wave number range of approx. 1000-3000.

In the absorption spectrum according to Figure 1, from which the effect of the water in the background has been eliminated, the absorption peak 1 of the C-H bond is seen at a wave number of approx. 2870, the absorption peak 2 of the C=O double bond at a wave number of approx. 1750, the absorption peak 3 of the C-C bond at a wave number of approx. 1440, and the absorption peak 4 of the single C-O bond at a wave number of approx. 1175. In addition to the absorption peaks of these bonds belonging to the fat molecule, the absorption peak of the C-OH bond of lactose at a wave number of approx. 1050 (at a wavelength of approx. 9.5 μm) is indicated in Figure 1 by reference numeral 5.

In the absorption spectrum of oleic acid triglyceride according to Figure 2, the same absorption peaks 1-4 due to the different bonds of fat as in Figure 1 are seen. In the absence of other absorbing constituents and scatter, the said peaks are in this case of a more or less equal value as the basis for quantitative determination of fat.

According to the basic idea of the invention, the concentration of fat in milk is determined from the absorption peak of the single C-O bond, seen in Figure 1 within a wave number range of 1060-1090, by subtracting from this the absorption at the reference wave number, which may be, for example, the wave number 1200, and by calculating the fat concentration by using a

formula having the form

$$F=ax^3 +bx^2 +cx-d$$

where F is the fat concentration and x = absorbance, and possibly by further making, in the result obtained, a correction due to the lactose peak 5. When a DaiLab IR-2000 spectrometer is used, the said calculation formula is

$$F=35.1x^3 +28.4x^2 +26.0x-0.02$$

and the lactose correction coefficient, by which the absorbance of the peak 5 is multiplied and then added to the fat concentration calculated from the C-O absorbance peak 4, is 0.203.

The significance of homogenization in the quantitative determination of milk fat was tested by measurements using a fixed-lattice DaiLab IR-2000 spectrometer, in which the separate detectors are located at specific points corresponding to the different wavelengths to be measured and in which all the absorbance measurements are performed simultaneously. In the said apparatus it is possible to measure all the absorbances within a short time period 0-140 times in succession and to calculate from their mean the fat concentrations by using predetermined arithmetic formulae.

The following table shows the fat concentrations determined from 10 different milk samples on the basis of the absorption peaks of different bonds in the fat molecule, in each case as the mean of 100 successive individual absorbance measurements. For comparison, each sample was measured both after homogenizer treatment and unhomogenized.

Table

Bond Wave- length	Sample	Determined fat concentration (%) Homogenized	Determined fat concentration (%) No homogenization
(C-O) 8.5 μm	1	3.42	3.42
	2	3.66	3.66
	3	3.42	3.41
	4	3.42	3.41
	5	4.30	4.29
	6	2.88	2.88
	7	3.72	3.73
	8	4.47	4.47
	9	4.13	4.12
	10	4.10	4.09
(C-C) 7.0 μm	1	3.43	3.43
	2	3.65	3.67
	3	3.43	3.43
	4	3.42	3.42
	5	4.31	4.31
	6	2.89	2.90
	7	3.74	3.76
	8	4.45	4.47
	9	4.11	4.12
	10	4.14	4.14
(C=O) 5.7 μm	1	3.42	3.38
	2	3.66	3.64
	3	3.41	3.40
	4	3.41	4.39
	5	4.30	4.21
	6	2.87	2.83
	7	3.73	3.70
	8	4.47	4.44
	9	4.11	4.08
	10	4.13	4.11

Table (continued)

Bond Wave- length	Sample	Determined fat concentration (%)	
		Homogenized	No homogenization
(C-H) 3.5 μm	1	3.44	3.48
	2	3.67	3.73
	3	3.41	3.47
	4	3.43	3.48
	5	4.40	4.42
	6	2.89	2.96
	7	3.73	3.84
	8	4.45	4.53
	9	4.11	4.20
	10	4.13	4.22

The results show that, when the determination is based on the absorption peak of the single C-O bond, the values obtained for the fat concentration within the framework of the reading precision of the apparatus are the same regardless of whether or not the sample has been homogenized before the absorption measurement. In practice the results shows that the homogenization of a sample of raw milk is unnecessary in the method according to the invention. For the sake of comparison it can be noted that also in the determination based on the absorption peak of the C-C bond the fat concentration values obtained from a homogenized sample and from an unhomogenized sample are very close to each other, although the correspondence is not as good as in the determination according to the invention, whereas in determinations based on the C=O absorption peak, and especially on the C-H absorption peak, the values obtained with a homogenized sample and an unhomogenized sample deviate from each other to such an extent that homogenization of the sample is indispensable in practical determinations.

It is clear for an expert in the art that the different embodi-

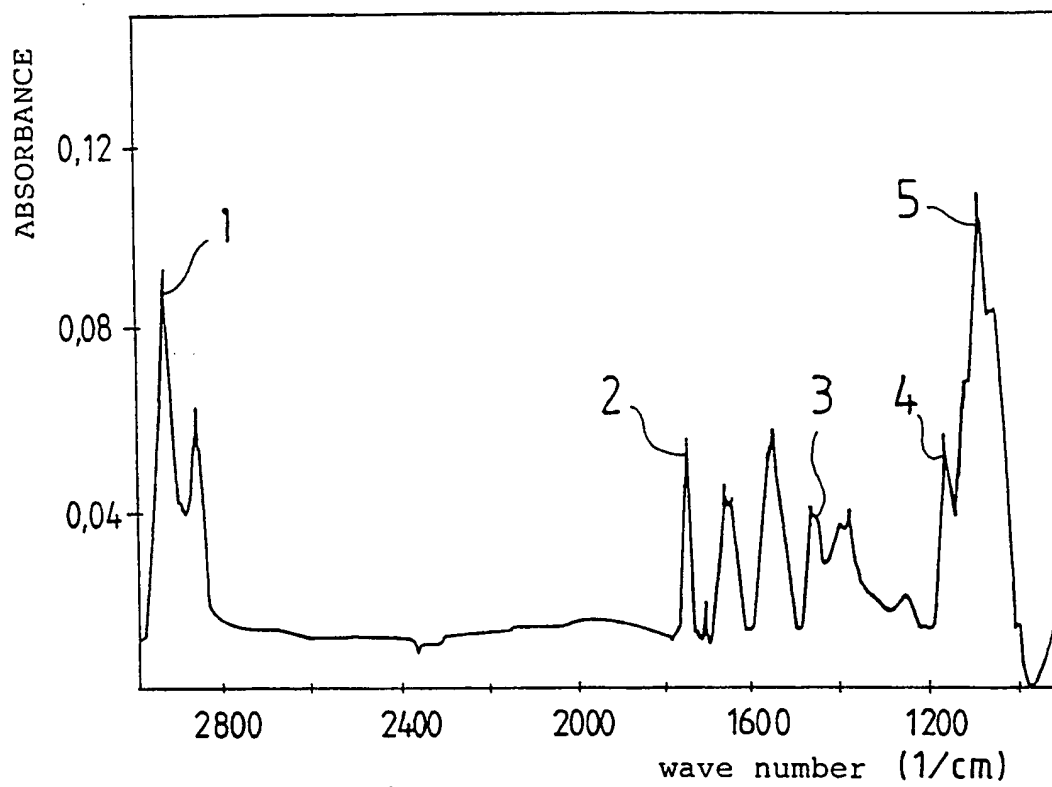
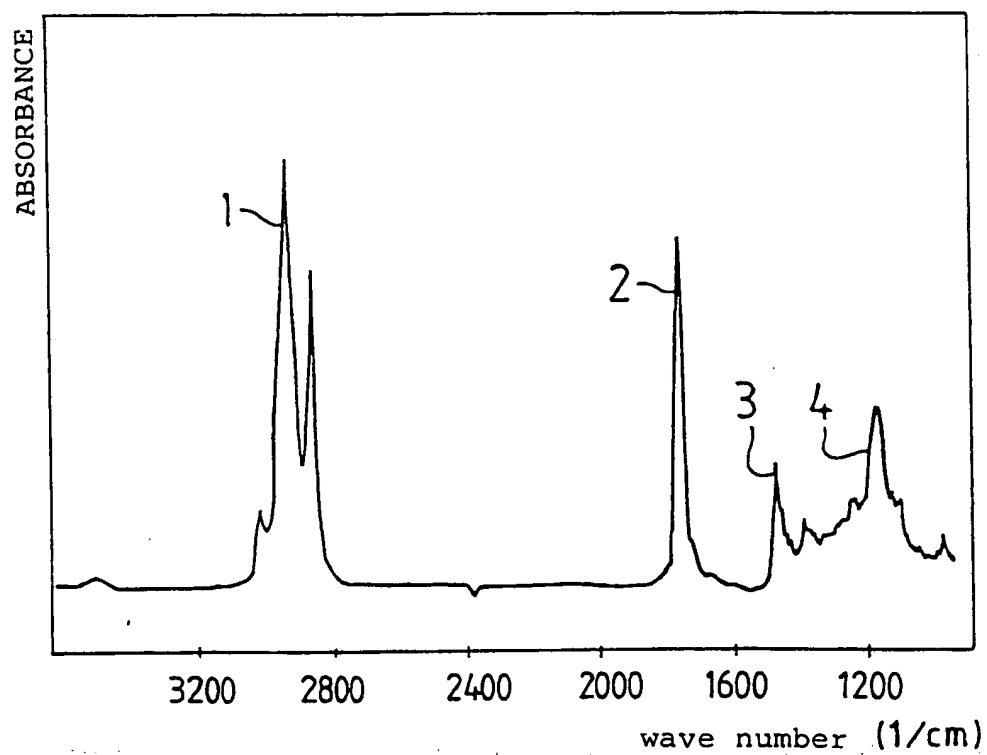
ments of the invention are not limited to that presented above as an example; they may vary within the scope of the accompanying patent claims. It should be noted in particular that the coefficients of the calculation formula presented are apparatus-specific values which change when a shift is made to the use of some other spectrometer. It should additionally be noted that, although the discussion above is mainly about the use of the invention in the determination of the fat concentration in milk or in milk products, emulsions containing other fats, such as vegetable fats, can also be measured in accordance with the invention.

Claims

1. A method for quantitative determination of fat from an emulsion containing fat particles, in which method the infrared absorption of an emulsion sample is measured and the fat is determined on the basis of a specific absorption peak in the absorption spectrum, by eliminating from it a any error effect possibly caused by other constituents, characterized in that the determination is carried out from the absorption peak, at a wave number of approx. 1160-1190 (1/cm), of single carbon-oxygen bonds (C-O) of the ester groups of the fat molecules.
2. A method according to Claim 1, characterized in that the reference used is the absorption measured at a wave number of approx. 1200 or at a wave number of approx. 1300-1350.
3. A method according to Claim 2, characterized in that a continuous infrared absorption spectrum is measured at least within a wave number range of approx. 1160-1350.
4. A method according to any of the above claims, characterized in that the emulsion is milk or a milk product.
5. A method according to Claim 4, characterized in that the emulsion is full milk having a fat concentration of approx. 3.0-5.5 %.
6. A method according to Claim 5, characterized in that the infrared absorption spectrum is measured from unhomogenized milk in which the fat particle size varies mainly within the range 0.5-5 μm .
7. A method according to any of Claims 4-6, characterized in that the absorption peak, at a wave number of approx. 1050, of the lactose present in the milk is measured, and on the basis of it the effect of lactose on the said absorption peak of the carbon-oxygen bond is eliminated.

8. A method according to Claim 7, characterized in that a continuous infrared absorption spectrum is measured within a wave number range of approx. 1050-1350.

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*Fig.1**Fig.2*

INTERNATIONAL SEARCH REPORT

International Application No PCT/FI 92/00100

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC5: G 01 N 21/35; 33/06		
II. FIELDS SEARCHED		
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III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	US, A, 4447725 (DELMAR A. BRIGGS ET AL) 8 May 1984, see abstract --	1-8
A	EP, A1, 0122749 (SHIELDS INSTRUMENTS LIMITED) 24 October 1984, see abstract --	1-8
A	US, A, 4310763 (JOHN SHIELDS) 12 January 1982, see abstract --	1-8
A	US, A, 4247773 (STEN A. NEXO ET AL) 27 January 1981, see abstract --	1-8
A	US, A, 4076983 (JOSEPH HOPKINS ET AL) 28 February 1978, see abstract -- -----	1-8
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IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
15th June 1992	1992 -07- 0 1	
International Searching Authority	Signature of Authorized Officer	
SWEDISH PATENT OFFICE	KARIN SÄFSTEN	

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ON INTERNATIONAL PATENT APPLICATION NO. PCT/FI 92/00100**

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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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